CASE REPORT

Novel *SOX9* Gene Mutation in Campomelic Dysplasia with Autosomal Sex Reversal

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Campomelic dysplasia (CD; OMIM #114290) is an autosomal dominant, frequently lethal dysplasia syndrome whose primary features include angular bowing and shortening of the limbs, and sex reversal in the majority of affected XY individuals. Most CD cases have heterozygous *de novo* mutations in the coding region of the transcription factor gene *SOX9* (*SRY*-related high-mobility group [HMG] box 9) in chromosome 17q. Here, we report a novel mutation of *SOX9* in a female neonate with CD with autosomal sex reversal. Respiratory distress and cyanosis were noted at birth, and endotracheal intubation with mechanical ventilation was performed due to respiratory failure. The presenting phenotypes included dysmorphic face with macrocephaly, prominent forehead, low nasal bridge, cleft palate and micrognathia. Skeletal deformities characteristic of CD were observed, including narrow thoracic cage, hypoplastic scapulae, scoliosis and short limbs with anterolateral femoral and tibial bowing. The karyotype was 46,XY despite female external genitalia. *SOX9* gene analysis revealed frameshift mutation (at nucleotide position 1095G → AT) in the open reading frame, resulting in a frameshift with 211 new amino acids. [*J Formos Med Assoc* 2006;105(12):1013–1016]

Key Words: autosomal sex reversal, campomelic dysplasia, *SOX9* gene

Campomelic dysplasia (CD; OMIM #114290) is an autosomal dominant, frequently lethal skeletal dysplasia syndrome whose primary features include angular bowing and shortening of the long bones (campomelia) with skin dimples, hypoplastic scapulae, missing pairs of ribs, a narrow thorax, and clubbed feet. Patients severely affected by CD usually die in the neonatal period due to respiratory distress resulting from Robin sequence, bell-shaped narrow thorax, hypoplastic lungs, and narrow airways. A secondary feature of CD is male-to-female sex reversal, which occurs in about two-thirds of XY karyotype patients. Like the sex reversal and the various skeletal symptoms, the bending of the long bones (campomelia) is not an obligatory feature and is absent in about 10% of cases, referred to as acampomelic CD (ACD).[1,2]

Most cases of CD are caused by heterozygous *de novo* mutations in *SOX9*, the gene encoding a member of the sex-determining region Y (*SRY*)-related high-mobility group box family of transcription factors located on human chromosome 17q24.3-q25.1.[3] *SOX9* contains a 79-amino acid DNA-binding motif known as the high-mobility-group (HMG) domain, which recognizes typical *SOX* binding sequences, and a second domain essential for its function, a proline/glutamine/serine-rich C-terminal transcription-activation domain.[4] The mutations cause loss of DNA binding or of the transactivation function of SOX9. Studies in mice have shown that *Sox9* functions as an

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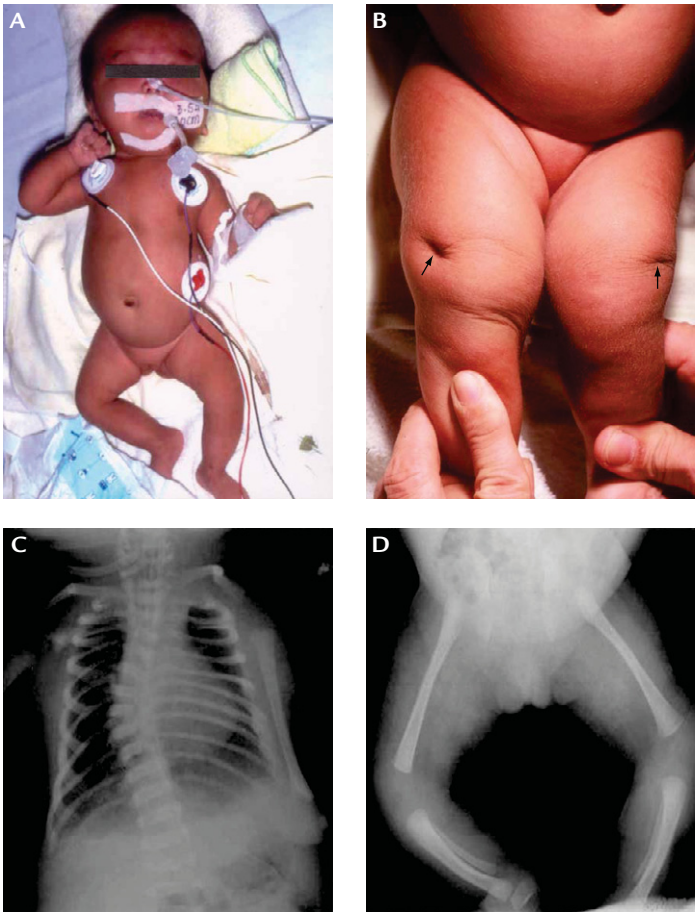

Figure 1. Clinical features of a campomelic dysplasia patient. (A) Macrocephaly, prominent forehead, low nasal bridge, micrognathia, and female external genitalia. (B) Skin dimples (arrows) on both thighs. (C) Chest X-ray shows bell-shaped, narrow thoracic cage, hypoplastic scapulae, and scoliosis. (D) Radiograph of bilateral legs shows anterolateral femoral and tibial bowing.

essential developmental regulator at various steps of chondrogenesis and during the initial phase of testis determination and differentiation.[5,6]

Here, we report a novel *SOX9* frameshift mutation in a Taiwanese neonate with CD and the characteristic features of autosomal sex reversal.

Case Report

A female neonate suffered from respiratory distress and cyanosis and was transferred to the neonatal intensive care unit 1 hour after birth. Emergent endotracheal intubation with mechanical ventilation was performed due to respiratory failure. This neonate was the first child born to nonconsanguineous Taiwanese parents without any family history of congenital malformations. The paternal and maternal ages were 29 and 28 years, respectively. The pregnancy course had been uneventful. The gestational age was 40 weeks and the birth body weight was 2780 g. Clinical features included dysmorphic face with macrocephaly, prominent forehead, low nasal bridge, cleft palate, micrognathia and female external genitalia (Figure 1A). Skeletal deformities included narrow thoracic cage with severe retraction sign and short limbs and skin dimples on the thighs (Figure 1B) with anterolateral femoral and tibial bowing and clubbed feet. Chest radiograph showed bell-shaped, narrow thoracic cage, hypoplastic scapulae, and scoliosis. Skeletal X-rays of bilateral legs showed anterolateral femoral and tibial bowing and ankle valgus deformity (Figures 1C, 1D). The karyotype was 46,XY despite female external genitalia. Abdominal sonography revealed a visible uterus and no evidence of testes. On the basis of characteristic clinical and radiologic features, CD with autosomal sex reversal was diagnosed. The patient died of respiratory failure at the age of 4 months.

To screen for a mutation, informed consent was obtained from the parents for blood sampling

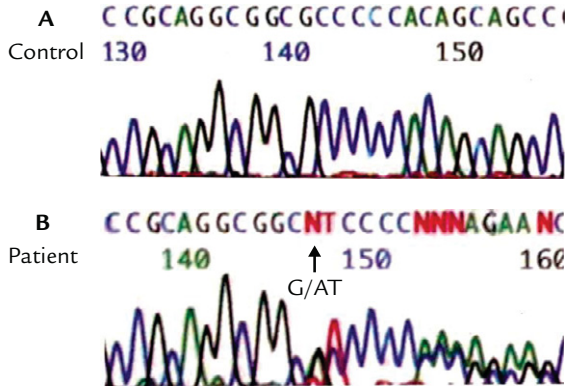

Figure 2. DNA sequencing of the *SOX9* gene of: (A) normal control and (B) the patient reveals a G to AT frameshift mutation at nucleotide 1095 (arrow) in the patient.

and the analysis was approved by the institutional review board of the hospital. The entire *SOX9* open reading frame was amplified from genomic DNA extracted from blood lymphocytes by polymerase chain reaction (using seven sets of primers). Direct automated sequencing (ABI 100) identified a novel G to AT frameshift mutation at nucleotide 1095 in exon 3, resulting in a frameshift with 211 new amino acids (forward: 5′-CCACGCACGGCCAGATCACC-3′; reverse: 5′-GTGCTGCTGCTGCTGGCTGTA-3′) (Figure 2). The patient carried a mutant allele and a normal allele, indicating that the mutation was heterozygous. Both parents declined genetic analysis.

Discussion

CD with autosomal sex reversal in an XY individual is a rare disease first reported by Bricarelli et al in 1981.[7] Most cases of CD are caused by *de novo* mutations in the *SOX9* gene located on chromosome 17q24.3-q25.1. There is a growing number of reports of CD with the chromosome 17 rearrangement breakpoint located some distance from *SOX9*.[3,8–10] Although CD with *SOX9* mutation has mainly been reported in North America, Europe and Australia, it has also been reported from Turkey and Japan.[11,12] This is the first report of CD with sex reversal in the Taiwanese population, and confirms the important role of the *SOX9* gene in this abnormality.

The *SOX9* gene encodes a member of the *SRY*-related HMG box family of transcription factors and is downstream of SRY in the male sex-determination cascade.[13] *SRY* is the gene encoding Y-linked testis-determining factor, and mutations in *SRY* also cause XY sex reversal but not CD.[14] On the contrary, heterozygous loss-of-function mutations in the *SOX9* gene have been identified in CD with XY sex reversal, identifying haploinsufficiency for SOX9 as the cause of both phenotypes in chondrogenesis and in testogenesis.[3,9] In the present case, the analysis in the open reading frame of the *SRY* gene in the Y chromosome did not reveal any mutation (data not shown), while the heterozygous mutation in the *SOX9* gene explained the phenotype of CD with sex reversal.

CD is a good model to understand how a single transcription factor can control the development of several organs. Both the skeletal dysplasia and the XY sex reversal in CD are caused by mutations in *SOX9*. All reported mutations in *SOX9* have been reported to cause CD, with approximately 75% associated with XY sex reversal, whereas no mutation in *SOX9* has been associated with isolated sex reversal.[15] The type and location of mutations in *SOX9* have not demonstrated any correlation with phenotypes. Bernard et al demonstrated that cooperative dimerization of *SOX9* was essential for activation of key chondrogenesis genes but not for male gonadal development, which may explain why CD is not necessarily associated with XY sex reversal.[16]

There are four major classes of heterozygous *SOX9* mutations causing CD: (1) amino acid substitutions in the HMG domain; (2) truncations or frameshifts that alter the C-terminus; (3) mutations at the splice junction; and (4) chromosomal translocations. All reported missense mutations lie in the HMG domain and affect DNA binding, frameshifts and splice mutations that truncate the C-terminus of *SOX9*, resulting in loss of transactivation domains.[15,16] Mutations in *SOX9* span the entire open reading frame, and no founder mutations have been reported. In Asian populations, *SOX9* missense mutations in codons 112 and 119, which lie in the HMG domain, have been

identified in Japanese and Turkish patients, respectively.[11,12] In the present case, a novel G to AT frameshift mutation was identified in codon 365 at nucleotide 1095 (G1095AT), resulting in a frameshift with 211 new amino acids. This mutation altered the transactivation domain in the C-terminus, leading to loss of function of *SOX9*.

In conclusion, apart from mutations in the *SRY* gene that cause XY gonadal dysgenesis, several autosomal and one-linked loci have also been associated with lack of testes determination. An autosomal locus *SOX9* on chromosome 17q24.3-q25.1 causes sex reversal and CD. We identified a novel *de novo* frameshift mutation (G1095AT) in the *SOX9* gene of a Taiwanese patient with CD and autosomal sex reversal. Further functional analysis of the HMG box, transactivation domain and dimerization mechanism is needed to clarify the genotype–phenotype relation to both skeletal development and testis determination.

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